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(57) Abstract

Compositions comprising Cu,Zn–Superoxide dismutase (Cu,Zn–SOD), nucleic acid encoding a Cu,Zn–SOD and/or antibody to a Cu,Zn–SOD are described as well as their use as vaccines. Also described are methods for isolation of Cu,Zn–SODs and for preparation of pharmaceutical compositions, preferably for providing or eliciting protective immunity to meningococcal infection in an animal.

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SUPEROXIDE DISMUTASE AS A VACCINE ANTIGEN

The present invention relates to pharmaceutical compositions for treating and/or vaccinating against bacterial infection and to methods of manufacturing such compositions. In particular, the invention relates to pharmaceutical compositions which comprise superoxide dismutase or antibodies thereto.

At present most bacterial infections in humans or animals are treated after infection has set in by administration of antibiotic drugs. As many more strains of pathogenic bacteria become resistant to current antibiotics, the range of options open for treatment decreases. Moreover, many antibiotics can cause dangerous side effects upon individuals or animals taking them, for example allergy to penicillin or the toxicity of sulpha drugs. Furthermore, antibiotic treatment is sometimes only effective if the drug is taken regularly over a period of time thus maintaining a constant level of therapeutic agent in circulation. If individuals forget or are unable to maintain the course of antibiotic treatment then it may be rendered ineffective.

Some bacterial infections progress very quickly, sometimes too quickly for antibiotic treatment to have much effect unless administered at a very early stage in the course of the infection. Meningococcal disease is one example of such a virulent infection and is caused by the pathogen *Neisseria meningitidis*. In many cases symptoms of disease at first resemble those of influenza and thus infected individuals often delay in seeking medical attention. Vaccines based on polysaccharides present on the surface of some *N. meningitidis* serogroups are available at present but they show limited protection against infection. Moreover, the surface polysaccharide of serogroup B strains of *N. meningitidis* (causative agent of over half the cases of meningococcal disease in the UK) are only weakly immunogenic and are not included in current vaccines.

Accordingly, it is an object of the invention to provide a pharmaceutical composition comprising a vaccine antigen or an antibody that effectively protects against or ameliorates bacterial infection. It is a further object of the invention to provide a pharmaceutical composition comprising a vaccine antigen that protects against meningococcal disease. It is yet a further object to provide a vaccine antigen that also provides protective immunity against a broader range of infectious bacteria. It is a further object to provide a method of manufacturing antibodies that can provide protective immunity to a range of bacterial pathogens when included in a pharmaceutical preparation. It is still a further object of the invention to provide a multivalent vaccine which provides protective immunity to a wide range of bacterial infections.

Cu,Zn-Superoxide Dismutase (Cu,Zn-SOD) is an metalloenzyme found in many prokaryotic and eukaryotic organisms. It catalyses the reduction of the superoxide radical anion, O_2^-, to hydrogen peroxide and molecular oxygen, thus playing an important role in the removal of cytotoxic free radicals from the organism. In bacteria Cu,Zn-SODs have been identified in the periplasm of a number of Gram negative species including *N. meningitidis*, *Haemophilus ducreyi*, *Haemophilus parainfluenzae*, *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* (Kroll et al, 1995). The enzyme can exist as a dimer or a monomer, and examples of monomeric Cu,Zn-SODS are those from *Brucella abortus* and *Escherichia coli* (Pesce, et al, 1997). It is believed that Cu,Zn-SOD provides a defence for the bacterium against the burst of oxygen free radicals released by phagocytic host cells, such as macrophages, during infection (Wilks et al, 1998; Farrant et al, 1997).

A known attempt to utilise Cu,Zn-SOD as a vaccine antigen has not been successful. Tabatabai (Tabatabai and Pugh, 1994) showed that a synthetic fragment of a monomeric Cu,Zn-SOD (denoted as peptide 3) from *B. abortus* was able to provide a low level of immunity in mice against *Brucella* infection, but the level of protection provided was lower than that seen when using

Brucella cell surface proteins and lipopolysaccharide antigens. Moreover, vaccination with the entire Cu,Zn-SOD failed to provide protective immunity at all. Tabatabai concluded that the antigenic fragment contained within *Brucella* Cu,Zn-SOD was counteracted by other parts of the protein which prevented it from eliciting an antigenic activity. This masking property was in fact so strong that even a mixture of synthetic peptides that included peptide 3 elicited no protective immunity to *Brucella* infection. Thus the study by Tabatabai et al teaches against the use of either fragments of Cu,Zn-SOD or full length Cu,Zn-SOD as an effective antigen that could provide protective immunity to bacterial infection.

Cu,Zn-SODs from eukaryotes and most Gram negative bacteria form dimers in their native form. However, the Cu,Zn-SODs of *E.coli* and *B. abortus* are atypical in that they are normally monomeric (Pesce et al, 1997). In all Gram negative bacteria that produce Cu,Zn-SOD the protein is localised to the periplasmic space between the outer cell wall and the cell membrane.

The present invention relates to the surprising discovery that a monoclonal antibody raised against a recombinant Cu,Zn-SOD fusion protein from *A. pleuropneumoniae* has bactericidal activity against *N. meningitidis* and protects mice against challenge with *N. meningitidis*. Furthermore, a recombinant, dimeric Cu,Zn-SOD and immunogenic fragments from *A. pleuropneumoniae* (a pig pathogen) act as antigen that can confer protective immunity not only against this organism but also against other Gram negative human pathogens such as *N. meningitidis*, *H. ducreyi*, *H. parainfluenzae* and *P. multocida*. Thus the Cu,Zn-SOD is suitable as a vaccine component against both animal and human bacterial diseases such as meningococcal disease (caused by *N. meningitidis*) or chancroid (caused by *H. ducreyi*), or porcine pleuropneumonia (caused by *A. pleuropneumoniae*).

Accordingly, a first aspect of the present invention provides a composition comprising a Cu,Zn-SOD of the dimeric type, or a fragment, variant or

derivative thereof, and a pharmaceutically acceptable carrier. The first aspect of the invention also provides a composition comprising a nucleic acid encoding a Cu,Zn-SOD of the dimeric type, or a fragment, variant or derivative thereof, and a pharmaceutically acceptable carrier. By "dimeric" we mean a SOD that naturally forms dimers under normal conditions, e.g. is found in dimeric form in nature. A pharmaceutically acceptable carrier could comprise an approved adjuvant such as alum or any other adjuvant approved for pharmaceutical purposes. The Cu,Zn-SOD can be from any bacteria, though especially from known pathogenic bacteria, and from *N. meningitidis* as an example.

The present invention is not to be restricted to the use of full length or wild type Cu,Zn-SOD of the dimeric type. An antigenic fragment of the Cu,Zn-SOD may also be used in a vaccine formulation. The fragment preferably comprises a region of the Cu,Zn-SOD that is on the surface of the protein, although any fragment that confers protective immunity to bacterial infection is suitable; and the term "fragment" is intended to encompass any fragment against which an antibody may be raised which antibody binds intact, full length SOD. Moreover, mutant variants which have been modified to increase antigenicity or fusion protein derivatives between all or a part of a Cu,Zn-SOD and another protein for the purposes of purification or increasing antigenicity may also be suitable for use in pharmaceutical compositions. Vaccine components of the invention also include derivatives and variants of Cu,Zn-SOD. The term "derivative" is intended to encompass combinations of Cu,Zn-SOD with other proteins or molecules, including carbohydrates to form conjugate vaccines, the derivative retaining antigenicity such that an antibody raised against the derivative binds intact, full length SOD. The term "variant" is intended to encompass a polypeptide having an amino acid sequence that varies from that of intact, full length SOD, but such that antibodies raised against the variant bind intact, full length SOD.

In a preferred embodiment of the invention the pharmaceutical composition

provides protection against meningococcal infection and/or disease. In further preferred embodiments of the invention the pharmaceutical composition provides protective immunity to infection from *Actinobacillus* species (e.g. *A. pleuropneumoniae, A. actinomycetemcomitans*), *Pasteurellaceae* species (e.g. *P. multocida*), *Neisseria* species (e.g. *N. meningitidis*), *Haemophilus* species (e.g. *H. influenzae, H. parainfluenzae, H. ducreyi*), *Escherichia coli*, *Salmonella* species and other bacteria producing a Cu,Zn-SOD. In a specific embodiment of the invention the Cu,Zn-SOD is expressed from a recombinant gene cloned from *Actinobacillus pleuropneumoniae*.

It is an advantage of the present invention that a Cu,Zn-SOD of the dimeric type, or a fragment, variant or derivative thereof, can confer protective immunity to infection from a broad range of bacterial pathogens. It is of further advantage that the present invention provides for pharmaceutical compositions comprising Cu,Zn-SODs, or a fragment, variant or derivative thereof, that are protective against bacterial infection in both humans and animals and in particular to meningococcal disease. It is of still further advantage that an antibody to a Cu,Zn-SOD from one species of bacteria can provide protective immunity to infection from a plurality of other species of bacteria, in particular that the invention provides protective immunity to meningococcal disease. A further advantage of the present invention is that Cu,Zn-SOD is relatively abundant and can be easily purified from bacterial cultures. Moreover, recombinant Cu,Zn-SODs can be fused to other proteins, such as glutathione-S-transferases, to facilitate purification from bacterial cultures expressing the fusion protein, and the Cu,Zn-SOD moiety retains both antigenicity and biological activity.

In use of the invention, a Cu,Zn-SOD is cloned from the pig pathogen *Actinobacillus pleuropneumoniae*, to give a recombinant form of the gene. The recombinant Cu,Zn-SOD gene is optionally linked to, such as by fusing, a glutathione-S-transferase gene to enable easy purification of the fusion protein when expressed in bacteria. A pharmaceutical preparation is prepared

comprising the purified Cu,Zn-SOD protein and a pharmaceutically acceptable carrier; in one use the carrier includes the adjuvant alum. The pharmaceutical composition is suitably administered to the individual via any route. The nature or form of the composition may be selected from any conventional pharmaceutical composition including but not limited to tablets, capsules, oral compositions, liquids, compositions for infusion, syrups, solutions, powdered formulations and granular formulations. The pharmaceutical composition, in use, stimulates the individual to produce antibodies against the antigenic Cu,Zn-SOD protein, some of which provide protective immunity to a broad range of pathogens. In particular the pharmaceutical composition provides protective immunity to meningococcal disease.

In a specific example of the invention in use, described in more detail below, a Cu,Zn-SOD gene - the *sodC* gene - is isolated from *N. meningitidis* genomic DNA by standard PCR techniques. The product of the PCR reaction additionally incorporates a His-tag sequence (coding for six histidines at the C terminus of the protein). The isolated *sodC* gene plus His-tag is cloned into an expression vector and then transformed into *E. coli*, where the protein product is expressed. The expressed protein is purified on a nickel charged affinity column, to which the His-tag preferentially binds. The SodC protein is then eluted from the affinity column and is suitably incorporated in the pharmaceutical composition of the invention as described above.

A second aspect of the invention provides a vaccine comprising a Cu,Zn-SOD, or a nucleic acid encoding a Cu,Zn-SOD, of the dimeric type, or a fragment, variant or derivative thereof.

In a specific embodiment of the invention the Cu,Zn-SOD of the dimeric type, or a fragment, variant or derivative thereof, is from a recombinant gene cloned from *Actinobacillus pleuropneumoniae*, though the invention also encompasses use of native proteins. In a further preferred embodiment the vaccine provides protective immunity against meningococcal meningitis.

Compositions and vaccines of the invention comprising a nucleic acid which encodes a Cu,Zn-SOD or fragment or derivative thereof are suitably prepared comprising the coding sequence inside a microparticle according to the methods of WO-A-97/17063, incorporated herein by reference.

A third aspect of the invention provides a method of preparing a pharmaceutical composition that consists of:-

1) cloning a gene for a Cu,Zn-SOD of the dimeric type to obtain a recombinant form of the gene; and

2) (a) synthesising Cu,Zn-SOD from the recombinant gene; and combining said Cu,Zn-SOD with a pharmaceutically acceptable carrier, or

(b) combining said gene with a pharmaceutically acceptable carrier.

A fourth aspect of the invention provides for a composition, especially a pharmaceutical preparation comprising an antibody to a Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant thereof, and a pharmaceutically acceptable carrier. It is optional, but not essential, that the antibody is a monoclonal antibody.

The present invention thus also provides for an antibody preparation that is raised against a dimeric Cu,Zn-SOD, or a fragment, derivative or variant thereof, from one species of Gram negative bacteria and that confers protective immunity to infection from this bacterium and also to infection from a plurality of other Gram negative bacteria. The antibody can be used in pharmaceutical preparations that confer passive immunity to bacterial infection upon a host organism. In the example described in more detail below a monoclonal antibody raised against Cu,Zn-SOD from *A. pleuropneumoniae* provides protection against *N. meningitidis* infection. Thus

the monoclonal antibody is suitable for use in treating acute cases of meningococcal disease as well as in providing passive immunity to future meningococcal disease.

In a specific embodiment of the invention the antibody provides protective immunity to meningococcal disease. Thus it is suitable for use in preparations that provide passive immunity to infection and also for treatment of individuals already suffering from meningococcal infection.

In further specific embodiments of the invention the antibody provides protective immunity to bacterial infection from a range of bacterial pathogens including *Actinobacillus pleuropneumoniae*, *Pasteurellaceae* species, *Neisseria* species and *Haemophilus* species.

In a further preferred embodiment of the invention the antibody displays bactericidal activity. This activity may be directly attributed to the antibody itself or mediated via the complement system.

A fifth aspect of the invention provides for a multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, wherein said plurality of Cu,Zn-SODs are from the same or different species of Gram negative bacteria. A specific embodiment of the invention provides a multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, and one or a number of different bacterial proteins, or a fragment, derivative or variant thereof, that is not or are not a Cu,Zn-SOD. A further preferred embodiment of the invention is a multivalent vaccine that provides protective immunity to meningococcal infection.

A sixth aspect of the invention provides for use of a Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant thereof, in the manufacture of a medicament, e.g. a vaccine, for use against bacterial

infection. In a preferred embodiment of the invention the use is in response to a bacterial infection due to Gram negative species of bacteria. In a specific embodiment of the invention the use is in response to a meningococcal infection. This aspect also provides a method of treating bacterial, particularly meningococcal, infection by administering an effective amount of a Cu,Zn-SOD or a fragment, variant or derivative thereof, or other composition according to the invention.

A seventh aspect of the invention provides for an antibody specific to bacterial Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant thereof. A preferred embodiment of the invention provides for a monoclonal antibody (MAb) that is specific to bacterial Cu,Zn-SOD.

There now follow examples of specific embodiments of the invention.

Example 1

Cu,Zn-SOD Monoclonal Antibody

Monoclonal antibodies (MAbs) were raised in mice against a glutathione-S-transferase fusion protein with the Cu,Zn-SOD from *Actinobacillus pleuropneumoniae*. The whole fusion protein as expressed in *E. coli* was found to be enzymically active. Of 72 potential hybridomas screened by western blotting, two recognised only *A. pleuropneumoniae* Cu,Zn-SOD, but one also recognised Cu,Zn-SOD *from H. parainfluenzae, H. ducreyi, N. meningitidis* and *A. actinomycetemcomitans*. The latter MAb was used for the passive protection studies.

Passive Protection

Three experiments were performed and all demonstrated that the MAb protects mice against lethal infections with *N. meningitidis*. Adult NIH mice were given an intra-peritoneal (ip) injection with antibody (50μl) per mouse 2h before challenge with *Neisseria meningitidis*. Mice were given further ip

injections of antibody 2,5 and 24h after the challenge. The *N. meningitidis* challenge dose was the stated number of viable bacteria (see tables of results), given as an 0.5ml ip injection, containing 2mg iron dextran. At 24h mice were given a further 2mg iron dextran in an 0.2ml ip dose. Mice were then observed for 72h and the health of the animals noted. The results of these experiments are shown in the following tables.

EXPERIMENT 1				
VACCINE	CHALLENGE DOSE	SURVIVORS / CHALLENGED		
		DAY 1	DAY 2	DAY 3
growth medium	10^5	5/5	4/5	4/5
growth medium	10^6	5/5	3/5	3/5
50μl MAb	10^5	5/5	5/5	5/5
50μl MAb	10^6	5/5	5/5	5/5
5μl MAb	10^5	5/5	5/5	5/5
5μl MAb	10^6	5/5	4/5	4/5
Polyclonal serum *	10^5	5/5	5/5	5/5
Polyclonal serum *	10^6	5/5	5/5	5/5

* - raised against meningococcal outer membrane vesicles.

EXPERIMENT 2				
VACCINE	CHALLENGE DOSE	SURVIVORS/CHALLENGED		
		DAY 1	DAY 2	DAY 3
Growth medium	2×10^5	5/5	5/5	5/5
Growth medium	2×10^6	5/5	1/5	0/5
50μl MAb	2×10^5	5/5	5/5	5/5
50μl MAb	2×10^6	5/5	5/5	5/5
5μl MAb	2×10^5	5/5	5/5	5/5
5μl MAb	2×10^6	5/5	3/5	3/5
Polyclonal serum *	2×10^5	5/5	5/5	5/5
Polyclonal serum *	2×10^6	5/5	5/5	5/5

* - raised against meningococcal outer membrane vesicles.

EXPERIMENT 3					
VACCINE	CHALLENGE DOSE	SURVIVORS / CHALLENGED			
		DAY 1	DAY 2	DAY 3	DAY 4
Growth medium	1×10^7	5/5	0/5	0/5	0/5
50μl MAb	1×10^7	5/5	4/5	3/5	2/5
5μl MAb	1×10^7	5/5	1/5	0/5	0/5
Polyclonal serum	1×10^7	5/5	1/5	0/5	0/5
50μl Yersinia MAb	1×10^7	5/5	1/5	0/5	0/5
5μl Yersinia MAb	1×10^7	5/5	0/5	0/5	0/5

Bactericidal Activity

In addition to passive protective activity, the Cu,Zn-SOD MAb has been found to be bactericidal to two strains of meningococcus, with titres of 64-128 compared to a titre of 256 for a Group B polysaccharide specific MAb.

Example 2

Cloning and Expression of Cu,Zn-SOD - Purification of protein via a nickel affinity column

Genomic DNA isolated from *N.meningitidis* strain MC58 was used as a template to amplify the *sodC* gene (Cu,Zn-SOD). Primers were designed using the information in the Genbank database. The 5' primer (SEQ ID NO.1)

incorporates an *Nde*I site at the level of the ATG start codon, allowing the PCR product to be cloned into a variety of pET (Novagen) and pMTL (CAMR) vectors for expression. The 3' primer (SEQ ID NO.2) generates a polyhistidine (6 x His) tag in the translated protein to facilitate nickel affinity purification. The primer sequences are as follows:

"SEQ ID NO.1" : 5' primer: 5' GGC ATA TGA ATA TGA AAA CCT TAT TAG 3'

"SEQ ID NO.2" : 3' primer: 5' GGG CTG AGC TTA TTA GTG GTG GTG GTG GTG GTG TTT AAT CAC GCC ACA TGC CAT ACG TG 3'

The products were sub-cloned into pET22b and transferred to BL21 DE3 for initial expression. His-tagged protein was purified on a nickel charged Hi-Trap column.

Example 3

Purification of protein via a glutathione affinity column

A glutathione-S-transferase (GST) fusion was generated by cloning the *sodC* gene of Example 2 (described above) into one of the pGEX series of vectors (Pharmacia). An N-terminal fusion was generated by cloning a *sodC* PCR product as an *Eco*RI-*Xho*I fragment in frame into pGEX-4T-1 (restriction sites generated by PCR primers containing these sites in-frame immediately upstream or downstream of the *sodC* coding sequence, including 3' stop codon). The expressed protein was a GST-SodC fusion linked by a thrombin cleavage site. The recombinant protein was purified on a glutathione affinity column and cleaved with thrombin to release SodC. This SodC protein has an additional five amino acids on the N terminus, compared to wild type protein (sequence GSPQF).

Example 4

Purification of protein via a glutathione affinity column - Alternative method

This alternative cloning strategy varies from the method of Example 3 in that it allows reduction in the number of additional N terminal amino acids on the cleaved SodC product. This is achieved by removing the internal MC58 *sodC* *Bam*HI restriction site by site directed mutagenesis. PCR amplification of this altered gene with a 5' *Bam*HI site in-frame upstream of the ATG start codon facilitates cloning of a *Bam*HI-*Eco*RI fragment into pGEX-2T vector (Pharmacia). The resultant GST-SodC fusion protein is expressed and SodC released by cleavage with thrombin protease, giving rise to SodC product with just a two amino acid N terminal 'tag' (sequence GS).

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CLAIMS:

1. A pharmaceutical composition comprising (i) a Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a fragment, variant or derivative thereof, or (ii) a nucleic acid coding therefor, and a pharmaceutically acceptable carrier.

2. A pharmaceutical composition according to Claims 1, wherein said composition provides protection against meningococcal infection.

3. A pharmaceutical composition according to Claim 1 or 2, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

4. A pharmaceutical composition according to Claims 1 or 2, wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

5. A pharmaceutical composition according to any previous claim, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

6. A vaccine comprising (i) a Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a fragment, variant or derivative thereof, or (ii) a nucleic acid coding therefor.

7. A vaccine according to Claim 6, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

8. A vaccine according to Claims 6 or 7, wherein said vaccine provides protection against meningococcal infection.

9. A method of preparing a pharmaceutical composition comprising:-

1) cloning a gene for a Cu,Zn-SOD of the dimeric type to obtain a recombinant form of the gene; and

2) (a) synthesising Cu,Zn-SOD from the recombinant gene; and combining said Cu,Zn-SOD with a pharmaceutically acceptable carrier, or

(b) combining said gene with a pharmaceutically acceptable carrier.

10. A pharmaceutical preparation comprising an antibody to a Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant thereof, and a pharmaceutically acceptable carrier.

11. A pharmaceutical preparation according to Claim 10, wherein said antibody provides protective immunity to meningococcal disease.

12. A pharmaceutical preparation according to Claim 10, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

13. A pharmaceutical preparation according to Claim 10 or 11, wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

14. A pharmaceutical preparation according to any of Claims 10 to 13, wherein said antibody displays bactericidal activity.

15. A multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, wherein said

plurality of Cu,Zn-SODS are from the same or different species of Gram negative bacteria.

16. A multivalent vaccine comprising a Cu,Zn-SOD of the dimeric type, or fragments, derivatives or variants thereof, and a second protein, or a fragment, derivative or variant thereof, that is not a Cu,Zn-SOD.

17. A multivalent vaccine according to Claims 15 or 16, wherein said vaccine provides protective immunity to meningococcal disease.

18. Use of a Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant thereof, in the manufacture of a medicament for treatment or prevention of bacterial infection.

19. Use according to Claim 18, wherein the bacterial infection is due to Gram negative species of bacteria.

20. Use according to Claim 18 or 19 wherein the bacterial infection is due to meningococcal infection.

21. An antibody specific to bacterial Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant thereof.

22. A monoclonal antibody according to Claim 21.

23. Use of a nucleic acid encoding a Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant thereof, in the manufacture of a vaccine against bacterial infection.

24. A method of treating or preventing bacterial infection comprising administering an effective amount of a Cu,Zn-SOD or fragment, variant or derivative thereof.

SEQUENCE LISTING

<110> Microbiological Research Authority
Imperial College School of Medicine
Gorringe, Andrew Richard
Langford, Paul Richard
Kroll, John Simon
Robinson, Andrew

<120> SUPEROXIDE DISMUTASE AS A VACCINE ANTIGEN

<130> 20860pct camr

<140>
<141>

<160> 2

<170> PatentIn Ver. 2.1

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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

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<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 2
gggctgagct tattagtggt ggtggtggtg gtgtttaatc acgccacatg ccatacgtg 59

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/02828

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/53 C12N9/02 A61K38/44 A61K31/70 A61K48/00
C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LANGFORD P R ET AL: "Cloning and molecular characterization of Cu,Zn superoxide dismutase from Actinobacillus pleuropneumoniae." INFECTION AND IMMUNITY, (1996 DEC) 64 (12) 5035-41. , XP000867122 page 5040, right-hand column, paragraph 2; figures 3-7; table 1 --- -/--	1-9, 18-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 January 2000	04/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Espen, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEAMAN L ET AL: "Monoclonal antibodies demonstrate that superoxide dismutase contributes to protection of Nocardia asteroides within the intact host" INFECTION AND IMMUNITY, vol. 58, no. 9, September 1990 (1990-09), pages 3122-3128, XP002127736 WASHINGTON US	21,22
Y	table 1	1-9, 18-20, 23,24
Y	TABATABAI L B: "Modulation of immune responses in Balb/c mice vaccinated with Brucella abortus Cu-Zn superoxide dismutase synthetic peptide vaccine" VACCINE, vol. 12, no. 10, 1994, pages 919-924, XP000867119 GUILDFORD GB abstract; figures 1-3	1-9, 18-24
Y	LANGFORD P R ET AL: "Distribution, cloning, characterisation and mutagenesis of sodC, the gene encoding copper/zinc superoxide dismutase, a potential determinant of virulence, in Haemophilus ducreyi." FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, (1997 APR) 17 (4) 235-42. , XP000866521 abstract; figure 2	1-9, 18-24
Y	WILKS K E ET AL: "Periplasmic superoxide dismutase in meningococcal pathogenicity." INFECTION AND IMMUNITY, (1998 JAN) 66 (1) 213-7. , XP000867120 the whole document	1-9, 18-24
Y	FARRANT J L ET AL: "Bacterial copper- and zinc-cofactored superoxide dismutase contributes to the pathogenesis of systemic salmonellosis." MOLECULAR MICROBIOLOGY, (1997 AUG) 25 (4) 785-96. , XP000866453 abstract	1-9, 18-24
Y	PATENT ABSTRACTS OF JAPAN vol. 016, no. 283 (C-0955), 24 June 1992 (1992-06-24) & JP 04 074134 A (FUJISAWA PHARMACEUT CO LTD;OTHERS: 01), 9 March 1992 (1992-03-09) abstract	1-9, 18-24

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Section Ch, Week 199252 Derwent Publications Ltd., London, GB; Class B04, AN 1992-430060 XP002128008 & JP 04 327541 A (NIPPON KAYAKU KK), 17 November 1992 (1992-11-17) abstract	1-9, 18-24
Y	DE 40 38 563 A (GRUENENTHAL GMBH) 11 June 1992 (1992-06-11) the whole document	1-9, 18-24
Y	WO 92 19224 A (OREAL) 12 November 1992 (1992-11-12) the whole document	1-9, 18-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/02828

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 24 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/02828

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 04074134 A	09-03-1992	NONE	
JP 4327541 A	17-11-1992	NONE	
DE 4038563 A	11-06-1992	AT 131065 T	15-12-1995
		DE 59107031 D	18-01-1996
		EP 0493662 A	08-07-1992
		HK 1005168 A	24-12-1998
		JP 4275231 A	30-09-1992
		US 5362492 A	08-11-1994
WO 9219224 A	12-11-1992	FR 2675997 A	06-11-1992
		AT 156702 T	15-08-1997
		CA 2109424 A	04-11-1992
		DE 69221640 D	18-09-1997
		DE 69221640 T	12-02-1998
		EP 0658099 A	21-06-1995
		ES 2104920 T	16-10-1997
		JP 6507165 T	11-08-1994
		US 5352438 A	04-10-1994

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
GWS/DC/20860		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 02828	27/08/1999	27/08/1998
Applicant		
MICROBIOLOGICAL RESEARCH AUTHORITY et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. -

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/02828

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 24 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]T/GB 99/02828

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/53 C12N9/02 A61K38/44 A61K31/70 A61K48/00
C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LANGFORD P R ET AL: "Cloning and molecular characterization of Cu,Zn superoxide dismutase from Actinobacillus pleuropneumoniae." INFECTION AND IMMUNITY, (1996 DEC) 64 (12) 5035-41. , XP000867122 page 5040, right-hand column, paragraph 2; figures 3-7; table 1 --- -/--	1-9, 18-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 January 2000	04/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Espen, J

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONS[redacted]ED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BEAMAN L ET AL: "Monoclonal antibodies demonstrate that superoxide dismutase contributes to protection of Nocardia asteroides within the intact host" INFECTION AND IMMUNITY, vol. 58, no. 9, September 1990 (1990-09), pages 3122-3128, XP002127736 WASHINGTON US	21,22
Y	table 1	1-9, 18-20, 23,24
Y	TABATABAI L B: "Modulation of immune responses in Balb/c mice vaccinated with Brucella abortus Cu-Zn superoxide dismutase synthetic peptide vaccine" VACCINE, vol. 12, no. 10, 1994, pages 919-924, XP000867119 GUILDFORD GB abstract; figures 1-3	1-9, 18-24
Y	LANGFORD P R ET AL: "Distribution, cloning, characterisation and mutagenesis of sodC, the gene encoding copper/zinc superoxide dismutase, a potential determinant of virulence, in Haemophilus ducreyi." FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, (1997 APR) 17 (4) 235-42. , XP000866521 abstract; figure 2	1-9, 18-24
Y	WILKS K E ET AL: "Periplasmic superoxide dismutase in meningococcal pathogenicity." INFECTION AND IMMUNITY, (1998 JAN) 66 (1) 213-7. , XP000867120 the whole document	1-9, 18-24
Y	FARRANT J L ET AL: "Bacterial copper- and zinc-cofactored superoxide dismutase contributes to the pathogenesis of systemic salmonellosis." MOLECULAR MICROBIOLOGY, (1997 AUG) 25 (4) 785-96. , XP000866453 abstract	1-9, 18-24
Y	PATENT ABSTRACTS OF JAPAN vol. 016, no. 283 (C-0955), 24 June 1992 (1992-06-24) & JP 04 074134 A (FUJISAWA PHARMACEUT CO LTD;OTHERS: 01), 9 March 1992 (1992-03-09) abstract	1-9, 18-24

-/--

2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/02828

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Section Ch, Week 199252 Derwent Publications Ltd., London, GB; Class B04, AN 1992-430060 XP002128008 & JP 04 327541 A (NIPPON KAYAKU KK), 17 November 1992 (1992-11-17) abstract	1-9, 18-24
Y	DE 40 38 563 A (GRUENENTHAL GMBH) 11 June 1992 (1992-06-11) the whole document	1-9, 18-24
Y	WO 92 19224 A (OREAL) 12 November 1992 (1992-11-12) the whole document	1-9, 18-24

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
T/GB 99/02828

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
JP 04074134	A	09-03-1992	NONE		
JP 4327541	A	17-11-1992	NONE		
DE 4038563	A	11-06-1992	AT	131065 T	15-12-1995
			DE	59107031 D	18-01-1996
			EP	0493662 A	08-07-1992
			HK	1005168 A	24-12-1998
			JP	4275231 A	30-09-1992
			US	5362492 A	08-11-1994
WO 9219224	A	12-11-1992	FR	2675997 A	06-11-1992
			AT	156702 T	15-08-1997
			CA	2109424 A	04-11-1992
			DE	69221640 D	18-09-1997
			DE	69221640 T	12-02-1998
			EP	0658099 A	21-06-1995
			ES	2104920 T	16-10-1997
			JP	6507165 T	11-08-1994
			US	5352438 A	04-10-1994

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

SCHLICH, George W.
MATHYS & SQUIRE
100 Gray's Inn Road
London WC1X 8AL
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	28.11.2000
Applicant's or agent's file reference GWS/DC/20860	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/02828	27/08/1999	27/08/1998

Applicant
MICROBIOLOGICAL RESEARCH AUTHORITY et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Emslander, S Tel.+49 89 2399-8718

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

REC'D 30 NOV 2000
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

15

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
GWS/DC/20860		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/02828	27/08/1999	27/08/1998
International Patent Classification (IPC) or national classification and IPC C12N15/53		
Applicant MICROBIOLOGICAL RESEARCH AUTHORITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/03/2000	28.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer van Heusden, M Telephone No. +49 89 2399 8145

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/02828

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-17 as originally filed

Claims, No.:

1-27 with telefax of 19/09/2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-5, 11-13, 15-27
	No:	Claims	6-10, 14
Inventive step (IS)	Yes:	Claims	1-5, 18-27
	No:	Claims	6-17
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	27

2. Citations and explanations
see separate sheet

Additional remarks to section V:

1. Citations

The documents mentioned in this IPER are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc. Furthermore, the following document, provided by the applicant, is cited:

D11: Alcendor et al. (1995) Gene, vol 164, pp 143-147.

2. Novelty (Article 33(2) PCT)

2.1 The present application discloses pharmaceutical compositions, vaccines and multivalent vaccines comprising bacterial Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a nucleic acid coding therefore. It further relates to the use of an antibody specific to bacterial Cu,Zn-SOD of the dimeric type and to a pharmaceutical preparation comprising an antibody to bacterial Cu,Zn-SOD of the dimeric type.

2.2 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject matter of claims 6-10 and 14 is not novel with regard to documents D1 and D2.

2.3 Claims 1-5, 'a pharmaceutical composition for vaccination, comprising ...' are interpreted as first medical use claims restricted to the composition used for vaccination, rather than as product claims of the pharmaceutical composition per se. Document D1 discloses the cloning and expression of a bacterial Cu,Zn-SOD of the dimeric type, i.e. the Cu,Zn-SOD of *Actinobacillus pleuropneumoniae* (see p. 5037, right column, l. 41-42). However, D1 does not disclose any medical use thereof. None of the further cited documents discloses a bacterial Cu,Zn-SOD of the dimeric type used for vaccination.

2.4 The subject matter of claims 6-8 relate to 'a vaccine comprising ...'. Thus these

claims are product claims. The wording 'vaccine comprising ...' is interpreted as a preparation of the bacterial Cu,Zn-SOD that elicits an immune response when injected into an animal, e.g. a human. Thus a preparation of a bacterial Cu,Zn-SOD in saline falls within the scope of these claims. D1 discloses a bacterial Cu,Zn-SOD of the dimeric type, also recombinantly expressed. Thus D1 anticipates the subject matter of claims 6-8.
Similarly, claim 9 is also anticipated by D1, which also discloses a method comprising the isolation of a gene for a bacterial Cu,Zn-SOD of the dimeric type, the (recombinant) synthesis of said SOD (Figure 5) and the combining with a pharmaceutically acceptable carrier (buffer).

2.5 The subject matter of claims 10 and 14 is not novel with regard to document D2, which discloses monoclonal antibodies to SOD of *Nocardia asteroides*. Although the SOD of *N. asteroides* may differ from the bacterial Cu,Zn-SOD of the present application in that the preferred metal ligand is Mn, this preference is based on the identity of a few amino acid residues only (D11: p. 145, left column, paragraph 1). Moreover, D11 discloses the *N. asteroides* SOD as binding equal molar amounts of Mn and Zn (p. 144, left column, paragraph 1). Thus said SOD may well have significant sequence similarity to the SOD of the present application. Thus, in the absence of the indication that the antibodies are specific for the bacterial Cu,Zn-SOD of the dimeric type, the monoclonal antibodies against *N. asteroides* SOD as disclosed in D2 anticipate the antibodies referred to in claim 10. The antibodies disclosed in D2 are described in sterile saline and disclosed to be injected into mice (p. 3123, last paragraph of left column - first paragraph of right column). Furthermore the antibodies have bactericidal activity as evident from figures 3 and 4 and the abstract. Thus D2 anticipates the subject matter of claims 10 and 14.

3. Inventive step (Article 33(3) PCT)

3.1 In the case that the subject matter of claims 10 and 14 would be restricted to an antibody specific to a bacterial Cu,Zn-SOD of the dimeric type, claims 10 and 14 would not be inventive in view of D1. D1 discloses a bacterial Cu,Zn-SOD of the dimeric type. To provide an antibody specific for said known Cu,Zn-SOD does not involve an inventive step. The pharmaceutical preparations according to claims 11-13 comprise the same antibody as the preparation according to claim 10. The

type of protective immunity provided by the pharmaceutical preparation merely describes a result which is achieved but does not render the pharmaceutical preparation per se inventive. Thus claims 10-14 lack an inventive step in view of D1.

3.2 The closest prior art to evaluate the inventiveness of the subject matter of claims 15-17 is also document D1. D1 anticipates the vaccine according to claims 6-8 (see above under item 2.4). Starting from the vaccine disclosed in D1, no inventive step is required to provide a multivalent vaccine by adding more Cu,Zn-SODs or to add any second protein to the vaccine. Thus claims 15-17 lack an inventive step in view of D1.

3.3 The closest prior art to evaluate the inventiveness of claims 1-5 and 18-27 is document D1. The subject matter of claims 1-5 and 18-27 differs from the subject matter disclosed in D1 in that the bacterial Cu,Zn-SOD of the dimeric type is used for medical applications such as a pharmaceutical composition for vaccination or manufacture of a medicament for treatment of bacterial infection. Thus the problem to be solved by the present alleged invention appears to be to provide a medical application for said known Cu,Zn-SOD.
Document D1 states that the Cu,Zn-SOD is localized in the periplasm and that it is therefore unlikely that antibodies against said Cu,Zn-SOD are useful in preventing disease. Thus D1 teaches away from the use of Cu,Zn-SOD for therapeutic purposes. Furthermore, document D4 does not disclose any role of Cu,Zn-SOD in virulence. Documents D5 and D6 disclose that bacterial Cu,Zn-SOD of the dimeric type plays an important role in bacterial virulence. However, neither of these documents discloses the effect of antibodies to Cu,Zn-SOD on the bacterial virulence.
Thus, although a role of Cu,Zn-SOD in bacterial virulence has been demonstrated, the prior art does not provide any evidence that antibodies to the Cu,Zn-SOD provide bactericidal activity and thus have therapeutic value. In view of the localisation of the Cu,Zn-SOD in the periplasm (and thus not exposed on the bacterial surface) it would not be obvious for the skilled person to select the Cu,Zn-SOD as a vaccine candidate. This is even more so in view of the suggestion in D1 leading the skilled person away from the use of Cu,Zn-SOD as a vaccine candidate. Therefore the subject matter of claims 1-5 and 18-27, related

to the use of the known Cu,Zn-SOD in vaccination or medical treatment, is considered to involve an inventive step.

4. Industrial applicability (Article 33(4) PCT)

4.1 The subject matter of claims 1-26 appears to be industrially applicable.

4.2 The subject matter of claim 27 includes a method of treatment of the human or animal body and is thus excluded from examination by Article 34(4)(a)(i) PCT in combination with Rule 67(iv) PCT. For the assessment of this claim on the question whether they are industrially applicable, no unified criteria exist in PCT. The patentability can also be dependent upon the formulation of the claim. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment. The applicant is already informed that in the case of a European application, claim 27 does not seem to be allowable because 'methods of treatment of human or animal body by surgery or by therapy and diagnostic methods practised on the human or animal body shall not be regarded as inventions which are susceptible of industrial application'.

CLAIMS:

1. A pharmaceutical composition for vaccination, comprising:-

(i) a bacterial Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind intact full length Cu,Zn-SOD; or

(ii) a nucleic acid coding for the Cu,Zn-SOD fragment, variant or derivative; and

a pharmaceutically acceptable carrier.

2. A pharmaceutical composition according to Claim 1, wherein said composition provides protection against meningococcal infection.

3. A pharmaceutical composition according to Claim 1 or 2, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

4. A pharmaceutical composition according to Claims 1 or 2, wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

5. A pharmaceutical composition according to any previous claim, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

6. A vaccine comprising (i) a bacterial Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative

also bind intact full length Cu,Zn-SOD; or (ii) a nucleic acid coding for the bacterial Cu,Zn-SOD fragment, variant or derivative.

7. A vaccine according to Claim 6, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

8. A vaccine according to Claims 6 or 7, wherein said vaccine provides protection against meningococcal infection.

9. A method of preparing a pharmaceutical composition comprising:-

1) isolating a gene for a bacterial Cu,Zn-SOD of the dimeric type or a fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind the full length intact Cu,Zn-SOD; and

2) (a) synthesising the Cu,Zn-SOD fragment, variant or derivative from the gene; and combining said Cu,Zn-SOD, fragment, variant or derivative, with a pharmaceutically acceptable carrier, or

(b) combining said gene with a pharmaceutically acceptable carrier.

10. A pharmaceutical preparation comprising an antibody to a bacterial Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant of the Cu,Zn-SOD, wherein antibodies raised against said fragment, derivative or variant also bind intact full length Cu,Zn-SOD; and a pharmaceutically acceptable carrier.

11. A pharmaceutical preparation according to Claim 10, wherein said antibody provides protective immunity to meningococcal disease.

12. A pharmaceutical preparation according to Claim 10, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

13. A pharmaceutical preparation according to Claim 10 or 11, wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

14. A pharmaceutical preparation according to any of Claims 10 to 13, wherein said antibody displays bactericidal activity.

15. A multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, wherein antibodies raised against said fragments, derivatives or variants also bind intact full length Cu,Zn-SOD, and wherein said plurality of Cu,Zn-SODS are from the same or different species of Gram negative bacteria.

16. A multivalent vaccine comprising a bacterial Cu,Zn-SOD of the dimeric type, or fragments, derivatives or variants thereof, wherein antibodies raised against said fragments, derivatives or variants also bind intact full length Cu,Zn-SOD; and a second protein that is not a Cu,Zn-SOD.

17. A multivalent vaccine according to Claims 15 or 16, wherein said vaccine provides protective immunity to meningococcal disease.

18. Use of a bacterial Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant of the Cu,Zn-SOD, wherein antibodies raised against said fragment, derivative or variant also bind intact full length Cu,Zn-SOD; in the manufacture of a medicament for treatment or prevention of bacterial infection.

19. Use according to Claim 18, wherein the bacterial infection is due to Gram negative species of bacteria.

20. Use according to Claims 18 or 19 wherein the bacterial infection is due to meningococcal infection.

21. Use according to Claim 18, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

22. Use according to Claim 19, wherein said gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

23. Use of a nucleic acid encoding a bacterial Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant of the Cu,Zn-SOD, wherein antibodies raised against said fragment, derivative or variant also bind intact full length Cu,Zn-SOD, in the manufacture of a vaccine against bacterial infection.

24. Use of an antibody specific to bacterial Cu,Zn-SOD of the dimeric type, or a fragment of the antibody, in the manufacture of a medicament for treatment or prevention of bacterial infection.

25. Use according to Claim 24 wherein the antibody is a monoclonal antibody.

26. Use according to Claims 24 or 25 wherein the bacterial infection is due to meningococcal infection.

27. A method of treating or preventing bacterial infection comprising administering an effective amount of a bacterial Cu,Zn-SOD or fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind intact full length Cu,Zn-SOD.

CLAIMS:

1. A pharmaceutical composition comprising (i) a Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a fragment, variant or derivative thereof, or (ii) a nucleic acid coding therefor, and a pharmaceutically acceptable carrier.

2. A pharmaceutical composition according to Claims 1, wherein said composition provides protection against meningococcal infection.

3. A pharmaceutical composition according to Claim 1 or 2, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

4. A pharmaceutical composition according to Claims 1 or 2, wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

5. A pharmaceutical composition according to any previous claim, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

6. A vaccine comprising (i) a Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a fragment, variant or derivative thereof, or (ii) a nucleic acid coding therefor.

7. A vaccine according to Claim 6, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

8. A vaccine according to Claims 6 or 7, wherein said vaccine provides protection against meningococcal infection.

9. A method of preparing a pharmaceutical composition comprising:-

1) cloning a gene for a Cu,Zn-SOD of the dimeric type to obtain a recombinant form of the gene; and

2) (a) synthesising Cu,Zn-SOD from the recombinant gene; and combining said Cu,Zn-SOD with a pharmaceutically acceptable carrier, or

(b) combining said gene with a pharmaceutically acceptable carrier.

10. A pharmaceutical preparation comprising an antibody to a Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant thereof, and a pharmaceutically acceptable carrier.

11. A pharmaceutical preparation according to Claim 10, wherein said antibody provides protective immunity to meningococcal disease.

12. A pharmaceutical preparation according to Claim 10, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

13. A pharmaceutical preparation according to Claim 10 or 11, wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

14. A pharmaceutical preparation according to any of Claims 10 to 13, wherein said antibody displays bactericidal activity.

15. A multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, wherein said

plurality of Cu,Zn-SODS are from the same or different species of Gram negative bacteria.

16. A multivalent vaccine comprising a Cu,Zn-SOD of the dimeric type, or fragments, derivatives or variants thereof, and a second protein, or a fragment, derivative or variant thereof, that is not a Cu,Zn-SOD.

17. A multivalent vaccine according to Claims 15 or 16, wherein said vaccine provides protective immunity to meningococcal disease.

18. Use of a Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant thereof, in the manufacture of a medicament for treatment or prevention of bacterial infection.

19. Use according to Claim 18, wherein the bacterial infection is due to Gram negative species of bacteria.

20. Use according to Claim 18 or 19 wherein the bacterial infection is due to meningococcal infection.

21. An antibody specific to bacterial Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant thereof.

22. A monoclonal antibody according to Claim 21.

23. Use of a nucleic acid encoding a Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant thereof, in the manufacture of a vaccine against bacterial infection.

24. A method of treating or preventing bacterial infection comprising administering an effective amount of a Cu,Zn-SOD or fragment, variant or derivative thereof.